Distribution of IgA_1 and IgA_2 subclasses in normal bone marrow trephines and in trephines infiltrated by IgA producing multiple myeloma

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SUMMARY A series of 20 bone marrow trephines biopsy and necropsy specimens were stained for IgA_1 and IgA_2 activity, together with total IgA, by means of an indirect immunoperoxidase technique using murine monoclonal antibodies applied to paraffin sections. The specimens showed normal histology and had been taken from patients not known to be suffering from haematological or related systemic disease. The IgA_1, IgA_2, and total IgA containing cells were counted and expressed as a percentage of all nucleate cells in the marrow cavities. Remarkably constant percentages of and ratios between these cell types were found. The same was true for a further 10 trephines taken from patients undergoing staging procedures for epithelial malignancies, where the marrow histology was normal. The pooled mean percentage of IgA_1 containing cells from both groups was 1·18% of all cells, that for IgA_2 containing cells 0·18%, and for total IgA positive cells 1·41%. In addition, 12 trephines containing known IgA producing myeloma were examined. Of these, 11 contained IgA_1, the remainder contained IgA_2 subclass.

The distribution of the two subclasses of immunoglobulin A (IgA) has previously been studied in cell suspensions from lymphoid and related tissues.[1 2] The possibility that IgA_1 and IgA_2 could be shown in paraffin wax embedded tissue sections is of interest, as this is a less cumbersome technique than cell suspension analysis. As the distribution of these IgA subclasses may be important in, for example, immunological[1] or neoplastic disease we decided that a quantitative study of cells containing IgA_1 and IgA_2 in normal marrow trephine biopsies was appropriate. Paraffin wax embedded marrow trephines have previously been shown to be very well suited to immunohistochemical analysis.[3–7] Furthermore, quantitative analysis of Ig classes has been carried out in this context.[3]

In addition to a study of IgA subclass distribution in normal marrow trephines, the histological investigation of multiple myeloma secreting IgA is also of interest, as, to the best of our knowledge, quantitative studies of IgA subclass distribution have not yet been performed in situ on bone marrow trephines, using immunoperoxidase methodology.

Material and methods

BONE MARROW SPECIMENS

Bone marrow specimens were removed 24–48 hours after death from 20 patients. Patients were selected on the basis of being previously healthy and having been killed suddenly in road traffic accidents. Fifteen of the patients were male; five female. The mean age was 40 years. Furthermore, 10 specimens were examined from 10 patients who were undergoing staging procedures for epithelial malignancy, where the marrow trephine was morphologically normal. Six of these patients were male; four female. Their mean age was 55.

Twelve trephines were also examined, which had been taken from patients (five men, seven women) with established IgA producing multiple myeloma. The mean age was 53 years.

A thin (5 mm thick) slice of bone marrow was removed from the anterior iliac crest from necropsy cases and fixed at room temperature in 5% glacial acetic acid in 10% formalin: this mixture simultaneously fixes and enhances immunostaining.[3] For the staging and myeloma specimens, Jamshidi needle specimens were taken from the anteror iliac crest and similarly processed. Afterwards the specimens were
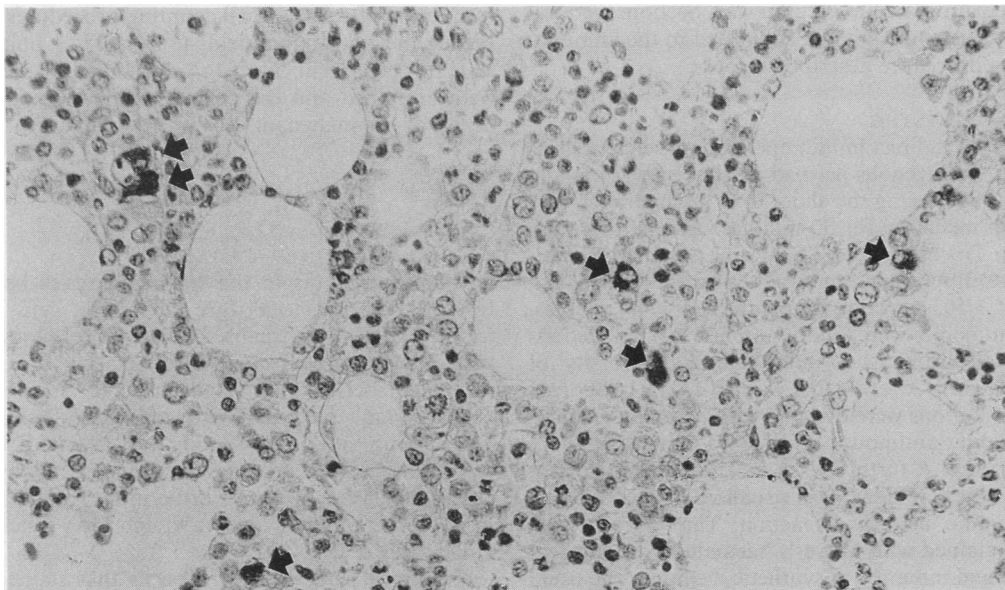
Fig 1 *Normal bone marrow trephine. Six IgA₁ subclass containing cells are present (arrows). (Peroxidase reaction with monoclonal antibody to IgA₁.) × 200.*
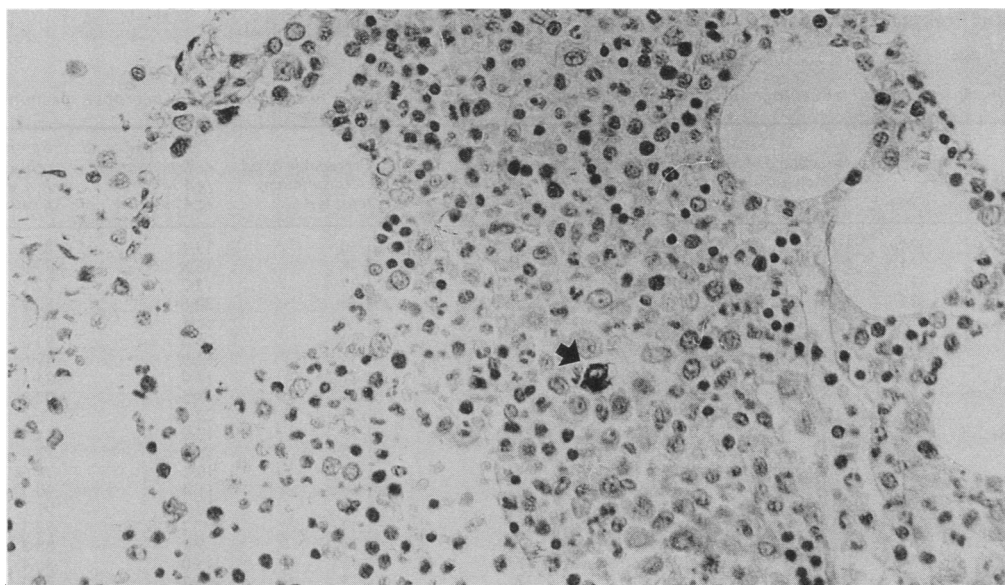

Fig 2 *Normal bone marrow trephine. One IgA₂ subclass containing cells is present (arrow). (Peroxidase reaction with monoclonal antibody to IgA₂.) × 200.*

routinely processed to paraffin wax. Sections were cut at a thickness of $2\,\mu m$ and submitted to the immunostaining procedure described below.

IMMUNOSTAINING

A standard indirect immunoperoxidase sequence was applied. Endogenous peroxidase activity was initially blocked by placing the slides in 0·3% hydrogen peroxide in methanol for 30 minutes. After a thorough wash in Tris buffer (pH 7·6) murine monoclonal antibodies to IgA_1, IgA_2, and total IgA were applied, at a titre of 1/50, at room temperature, for 40 minutes. (The antibodies were kindly supplied by Dr R Jefferis of the department of immunology, University of Birmingham). After further washes in Tris buffer (pH 7·6) the sections were overlaid with peroxidase conjugated rabbit antimouse IgM for 40 minutes at room temperature. A further wash in Tris buffer followed. Finally, the peroxidase was visualised by means of the 3·3'-diamino benzidine reaction. The sections were counterstained with Mayer's haemalum, dehydrated, cleared and mounted in synthetic medium. The usual controls, including adsorption studies, with appropriate IgA, IgA_1, and IgA_2 antigens, were performed.

COUNTING PROCEDURE

For each normal specimen, 1000 total cells were counted in the marrow spaces. For each of these thousand cells, those staining for IgA_1, IgA_2, or total IgA were enumerated and expressed as a percentage of all cells. A simple eyepiece graticule was used to avoid recounting of cells, and all counting procedures were carried out on random fields using a 25 × objective lens. For the specimens of IgA myeloma, the sections were examined and the subclass of IgA being produced by the malignant cells was determined.

Results

NORMAL TREPHINES—CADAVERS

In all instances (as in the staging biopsies below), IgA_1, IgA_2, and total IgA containing cells were readily and clearly visualised. Their morphology was that of mature, Marschalkò type plasma cells, and they often occurred in small clusters. Virtually no "background" reaction was evident (figs 1 and 2). The staining with each antibody was "blocked" by its corresponding IgA subclass antigen but not by the other subclass antigen. Furthermore, staining of serial sections did not show IgA_1 and IgA_2 subclasses in the same cell.

The mean percentage of all cells that stained for IgA_1 was 1·19. The corresponding percentage for IgA_2 containing cells was 0·19. The percentage of all cells containing total IgA was 1·37. The cells containing IgA_1 formed a mean of 85·7% of all cells staining for total IgA, and those containing IgA_2 comprised 13·6% of all total IgA containing cells. (table 1) The interval between the time of death and the removal of the marrow specimens did not affect the results of immunostaining.

Table 1 *Specimens studied and percentages of IgA_1, IgA_2 and total IgA containing cells in normal trephines (necropsy specimens)*

Specimen (normal trephines)	Age (years)	Sex	Percentage of all cells containing IgA_1	Percentage of all cells containing IgA_2	Ratio of IgA_2 positive: IgA_1 positive cells	Percentage of all cells containing total IgA	Percentage of all cells containing IgA which are IgA_2 positive	Percentage of all cells containing IgA which are IgA_1 positive
1	16	M	1·11	0·17	15	1·30	13·1	85·4
2	18	M	1·33	0·19	14	1·48	12·8	89·9
3	18	F	1·21	0·17	14	1·39	12·2	87·1
4	22	M	1·10	0·28	25	1·36	20·6	80·9
5	24	M	1·08	0·22	20	1·34	16·4	80·6
6	27	M	1·30	0·09	7	1·39	6·5	93·5
7	27	M	1·21	0·16	13	1·35	11·9	89·6
8	30	F	1·19	0·21	18	1·41	14·9	84·4
9	31	M	1·18	0·11	9	1·28	8·6	92·2
10	33	M	1·05	0·31	30	1·37	22·6	76·6
11	36	M	1·09	0·18	17	1·29	13·9	84·5
12	42	M	1·13	0·21	19	1·34	15·7	71·9
13	47	M	1·21	0·29	24	1·52	19·1	79·6
14	47	M	1·27	0·16	13	1·40	11·4	90·7
15	51	F	1·33	0·15	11	1·46	10·3	91·1
16	57	F	1·31	0·11	9	1·42	7·7	92·3
17	61	M	1·19	0·13	11	1·34	9·7	88·8
18	72	M	1·21	0·21	17	1·40	15·0	86·4
19	73	F	1·04	0·28	27	1·35	20·7	77·0
20	75	M	1·20	0·12	10	1·30	9·2	92·3
Mean	40		1·19	0·19	16	1·37	13·6	85·7
SD	18·7		0·09	0·06	6·26	0·062	4·4	6·03
SEM	4·29		0·02	0·014	1·44	0·014	1·02	1·38

Table 2 *Specimens studied and percentages of IgA₁, IgA₂, and total IgA containing cells in normal trephines (staging biopsies)*

Specimen	Age	Sex	Percentage of all cells containing IgA_1	Percentage of all cells containing IgA_2	Ratio of IgA_2 positive: IgA_1 positive cells	Percentage of all containing total IgA	Percentage of all cells containing IgA which are IgA_2 positive	Percentage of all cells containing IgA which are IgA_1 positive
1	44	M	1·10	0·16	15	1·41	11·3	78·0
2	44	F	1·12	0·18	16	1·37	13·1	81·8
3	48	F	1·14	0·19	17	1·52	12·5	75·0
4	49	M	1·21	0·16	13	1·46	11·0	82·9
5	49	M	1·10	0·22	20	1·39	15·8	79·1
6	50	F	1·28	0·24	19	1·40	17·1	91·4
7	51	M	1·15	0·18	16	1·30	9·2	88·5
8	58	M	1·20	0·12	10	1·71	7·1	70·2
9	62	F	1·19	0·16	13	1·41	11·3	84·4
10	63	M	1·22	0·14	7	1·38	10·1	88·4
Mean	52		1·17	0·17	17	1·44	11·85	81·9
SD	6·5		0·05	0·04	1·21	0·11	2·81	6·25
SEM	2·03		0·016	0·013	0·38	0·034	0·88	1·95

NORMAL TREPHINES—STAGING BIOPSIES

IgA₁ containing cells formed a mean of 1·17% of all cells and this mean percentage for IgA₂ containing cells was 0·17%. The percentage of all cells that stained for total IgA was 1·44. IgA₁ containing cells formed a mean of 81·9% of all total IgA containing cells, and the corresponding figure for IgA₂ was 11·85% (table 2). These data corresponded closely with those derived from necropsy tissue.

IGA MYELOMAS

In all specimens the marrow cavities were diffusely infiltrated by sheets of myeloma cells. These stained strongly for total IgA and IgA₁ or IgA₂ subclasses in all cases. Staining for both subclasses was not seen in any specimens (figs 3 and 4). Of the 12 specimens, 11 stained for IgA₁, the remaining case reacting for IgA₂.

Discussion

In man IgA exists as two antigenically different subclasses, IgA₁ and IgA₂. IgA₁ has been shown to account for about 90% of total serum IgA but it may
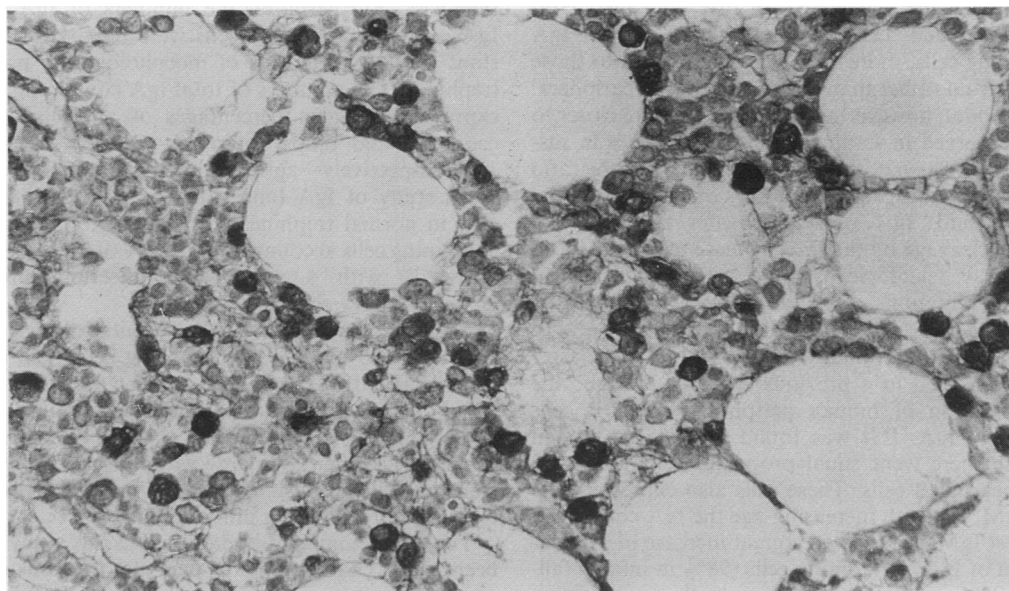

Fig 3 *Bone marrow infiltrated by IgA myeloma. Infiltrating cells contain IgA₁ (Peroxidase reaction with monoclonal antibody to IgA₁.) × 400.*
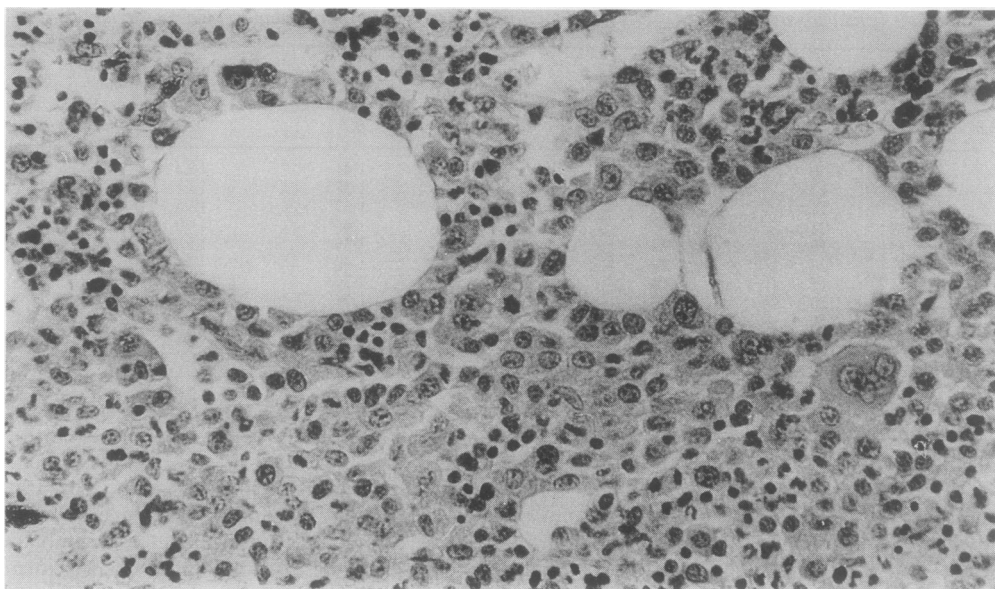
Fig 4   *Bone marrow infiltrated by IgA myeloma from same specimen as in fig 3. Cells do not contain IgA$_2$.*
(Peroxidase reaction with monoclonal antibody to IgA$_2$.) × 400.

be less predominant in other fluids, such as colostrum, where IgA$_1$ constitutes 70% or less of total IgA.[8] Using immunofluorescence on frozen sections, a previous study[1] showed that in mucosal and glandular tissues the percentages of IgA$_1$ and IgA$_2$ containing plasma cells, · in relation to total IgA containing cells, more closely approximated to those in colostrum rather than those in serum. In peripheral lymph nodes, however, the proportions were closer to those observed in serum and in marrow cells in suspension.[9] Even in normal subjects, therefore, the ratio of IgA$_1$:IgA$_2$ bearing cells is not constant for all tissues and fluids. IgA$_2$ possesses greater resistance than IgA$_1$ to cleavage by the IgA protease of streptococci, which may, teleologically, suggest that its biological role in secretions such as colostrum is highly important.[10]

Monoclonal antibodies to both IgA subclasses have been used in a previous study to examine the differentiation of human peripheral blood B cells expressing IgA.[11] It was found that in the human neonate there were equal proportions of IgA$_1$ and IgA$_2$ bearing B cells. These cells also expressed surface IgM, but with increasing age the IgA containing cells lost IgM and showed a great increase in the proportion of IgA$_1$ containing cells (98% in infants falling to 80% in adults) in relation to those expressing IgA$_2$.

Our study has shown that the three monoclonal

antibodies used can readily be applied to paraffin embedded tissue sections. They apparently do not cross react, as staining for IgA$_1$ and IgA$_2$ was not observed in the same cell in serial sections cut at 2 μm thickness. Furthermore, the numbers of IgA$_1$ and IgA$_2$ containing plasma cells were remarkably constant in the two groups of morphologically normal trephines. The numbers of total IgA containing cells, expressed as mean percentages of all cells in the cadaveric and biopsy marrows—namely, 1·37 and 1·44, respectively—agreed with the results of a previous study of IgA (and other Ig class) containing cells in normal trephines.[3] In normal trephines IgA containing cells accounted for 1·28% of all cells when "stained" with a polyclonal antiserum directed against α heavy chain.

The constancy of the current results could suggest that the ratio of IgA$_1$:IgA$_2$ bearing cells is in some way regulated in the normal marrow and is closely allied to the ratio seen in peripheral lymphoid tissues and serum. The same is true of the absolute numbers of these cells when compared with those of a previous study of cell suspensions of bone marrow and other lymphoid organs using immunofluorescence.[2]

The monoclonal antibodies used in this study have been highly characterised as part of a previous study of immunogenic and antigenic epitopes, including a range of antibodies specific for human IgA, IgA$_1$, and IgA$_2$ subclasses and an nA2m(2) isoallotypic epit-

ope.[12] They have also proved to be of great value in the study of IgA subclass distribution in normal and diseased lungs,[13] where IgA containing plasma cells (especially those with IgA₁) were shown to be greatly increased in chronic bronchitis.

The occurrence of IgA synthesis by multiple myeloma is well recognised and is seen in about 20% of all specimens,[14] being second in frequency to IgG secreting myelomas. The two subclasses of IgA were originally discovered in IgA myeloma[15] and, indeed, antibodies to IgA₁ and IgA₂ have generally been prepared against myeloma protein.[11] [12] We observed that most of our series of IgA myelomas were expressing IgA₁ rathen than IgA₂. This may reflect a simple probabilistic phenomenon, where IgA₁ containing cells, being more common that IgA₂ containing cells in healthy subjects, are also more likely to become malignant. Certainly, our results reflect those obtained serologically in patients with IgA myeloma.

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